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Sinogenomax Company Ltd. et al
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We hereby file additional claims 21-27 under Article 34(2) and wish them to be made subject of the detailed international preliminary examination requested in our previous letter of 12 January. Enclosed is also Form 1010 authorising the EPO to deduct the prescribed fee for the additional claims sheet exceeding 30 from our deposit account.

The content of the additional claims does not go beyond the disclosure of the international application as filed.

Additional claim 21 is an elucidation of present claim 8.
Accordingly, in line 2 of added claim 21 the at least two promoters have been specified to be "arranged in a convergent manner". Also, in line 4, the terminators have been specified to be "arranged in a convergent manner and in correlation to the promoters"

The convergent manner is clear from for instance the description under the heading, page 6 line 7 through page 8 line 7, and especially page 7 line 9: "Clone the above PCR product in between Sal I-Hind III, in a reversed orientation"; i.e. a second orientation, by which line it is understood that the promoters are arranged in a convergent manner when first having cloned the PCR-product in a first orientation (page 6, line 25), i.e. an orientation reverse to the second orientation. That the terminators are arranged in a convergent manner and in correlation to the promoters follows from for instance page 5, line 16 through line 23.

For the support of additional claim 22, reference is made to the above paragraph and the support of additional claim 21.

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For the support of additional claim 23, reference is made to the paragraph regarding the support of additional claim 21, and especially to lines 7-8 on page 6, which disclose a DNA-vector of additional claim 23.

Additional claim 24 is supported by page 5, lines 23-25.

Additional claim 25 and additional claim 26 are both supported by page 12, lines 12-14, from which lines it is clear that the promoters can be identical as well as different.

Finally, the individual nucleic acid construct of additional claim 27 are supported by for instance page 7 line 16 through page 8 line 7, and page 8, lines 2-7, clearly disclose a nucleic acid construct that expresses duplex RNA of proper length, i.e. 15-30 base pairs (supported by for instance present claim 3).

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Encls. Additional claims 21-27
Fee calculation sheet

21. The DNA library of any of claims 1-6 wherein each member of the library contains at least two promoters arranged in a convergent manner for transcription of the components of the double stranded RNA molecules, and two terminators arranged in a convergent manner and in correlation to the promoters for transcription of the components of the double stranded RNA molecules.

22. Each individual member of the DNA library of claim 21 wherein each such individual member contains at least two promoters arranged in a convergent manner for transcription of the components of the double stranded RNA molecules, and two terminators arranged in a convergent manner and in correlation to the promoters for transcription of the components of the double stranded RNA molecules.

23. A DNA vector for construction of the DNA library of claim 21 and the individual members thereof, wherein the vector contains at least two promoters arranged in a convergent manner for transcription of the components of the double stranded RNA molecules, and two terminators arranged in a convergent manner and in correlation to the promoters for transcription of the components of the double stranded RNA molecules.

24. A DNA vector of claim 23 wherein the said at least two promoters are substantially identical.

25. A DNA vector of any of claims 23-24 wherein said at least two promoters are RNA Polymerase III promoters.

26. A DNA vector of claim 23 wherein the said two promoters are substantially different.

27. An individual nucleic acid construct made from vectors of any of claims 23-26 for expressing double stranded RNA of a predefined duplex region in the length range of 15-30 base pairs.

CLAIMS

1. A DNA-library for production of a library of double stranded RNA-molecules (dsRNA) of a predefined length, the library consisting of double stranded DNA-molecules (dsDNA) where each dsDNA comprise a stretch wherein both strands contiguously encode a promoter, a dsRNA-encoding sequence of 10-30 base pairs encoding the dsRNA to be produced and a transcription termination sequence, wherein each of said promoters has been mutated to include the sequence complementary to the termination sequence of the other strand.

2. A DNA-library according to claim 1, wherein said promoters are H1-promoters or U6-promoters that have been mutated so as to incorporate an AAAAA-stretch at the end of the promoter, immediately next to the transcription starting site.

3. A DNA-library according to claim 1 or 2, wherein said dsRNA-encoding sequence is randomized in between 4 and all positions.

4. A DNA-library according to any of claims 1-3, wherein the produced dsRNA contains a single stranded region at one end.

5. A DNA-library according to any of claims 1-3, wherein the produced dsRNA contains single stranded regions at both ends.

6. A DNA-library according to claim 4 or 5, wherein at least one of the single stranded regions of the dsRNA is a poly-U overhang.

7. A DNA-library according to claims 4 or 5, wherein at least one of the single stranded regions of the dsRNA is a UU overhang.

8. A DNA-library according to any of claims 1-7, wherein it is constructed in a plasmid vector.

9. A DNA-library according to any of claims 1-7, wherein it is constructed in a viral vector.

10. A DNA-library according to any of claims 1-9, wherein the randomness of the library was modified by selection of the random DNA oligonucleotides, before cloning the said random DNA oligonucleotides into the vectors, through hybridization to a total RNA preparation or total mRNA preparation from a source, whereby only the oligonucleotides

hybridized to the source RNA (or mRNA) are subsequently cloned into the vector, and wherein the source can be a cell, a cell line, a tissue, or a organism.

11. A kit containing the DNA-library according to any of claims 1-10.

12. An RNA-library obtained from the DNA-library according to any of claims 1-10.

13. A method of using the DNA-libraries of any of the claims 1-10, wherein the library is transiently or permanently introduced into cells as a mixture.

14. A method of screening for double stranded RNA with biological functions comprising the use of the DNA-library according to any of claims 1-10 or the RNA-library according to claim 12.

15. A method of screening for novel genes comprising the use of the DNA-library according to claims 1-10 or the RNA-library according to claim 12.

16. An individual DNA-member of the DNA-library according to any of the claims 1-10.

17. An individual RNA-member of the RNA-library according to claim 12.

18. Use of a DNA-molecule comprising the DNA-sequence $AAAAA(N)_nTTTTT$, wherein $(N)_n$ is a randomized region of 19, 20 or 21 nucleotides, in the production of dsRNA-molecules.

19. An H1 RNA-polymerase III-promoter mutated to have an AAAAA-stretch at the end of the promoter immediately ahead of the transcription starting site.

20. A plasmid with two mutated RNA polymerase III promoters, each embedding one transcription termination sequence for the other promoter, and a siRNA-encoding region between the promoters.

21) Any polymerase III-promoter mutated to have an AAAAA-stretch at the end of the promoter immediately ahead of the transcription starting site.